

REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons which follow.


Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE TO SPECIFICATION

Marked up replacement paragraphs:

On page 6, delete the first paragraph under the heading "Brief Description of the Drawings," and replace this paragraph with the following:

Figure 1 presents DNA sequence (SEQ ID NO: 1) of an approximately 6 kb fragment that encodes β -glucuronidase from *Staphylococcus*.

On page 6, delete the third full paragraph under the "Brief Description of the Drawings," and replace this paragraph with the following:

Figures 3A-B present amino acid sequences (SEQ ID NOS 2-6) of representative microbial β -glucuronidases.

On page 6, delete the fourth full paragraph under the "Brief Description of the Drawings," and replace this paragraph with the following:

Figures 4A-J present DNA sequences (SEQ ID NOS 7-14) of representative microbial β -glucuronidases.

On page 6, delete the fifth full paragraph under the "Brief Description of the Drawings," and replace this paragraph with the following:

Figures 5A-C present amino acid alignments of *Staphylococcus* GUS (SGUS) (SEQ ID NO: 15) *E. coli* GUS (EGUS) (SEQ ID NO: 17) and human GUS (HGUS) (SEQ ID NO: 16) (5A). Microbial GUSes (5B) and nucleotide sequence alignments (SEQ ID NOS 18-23 and 24-26) of *Staphylococcus*, *Salmonella*, and *Pseudomonas* β -glucuronidases.

On page 7, delete the fourth full paragraph, and replace this paragraph with the following:

Figures 13A-C present a DNA sequence of *Staphylococcus* GUS (SEQ ID NO: 27 and 28) that is codon-optimized for production in *E. coli*.

On page 7, delete the seventh full paragraph, and replace this paragraph with the following:

Figure 16 is a DNA sequence of a *Salmonella* gene β --glucuronidase (SEQ ID NO: 113).

On page 7, delete the eighth full paragraph, and replace this paragraph with the following:

Figure 17 is an amino acid sequence of a *Salmonella* gene β --glucuronidase translated from the DNA sequence (SEQ ID NO: 114).

On page 7, delete the ninth full paragraph, and replace this paragraph with the following:

Figure 18A-C presents an alignment of amino acids of three β --glucuronidase gene products: Staph (*Staphylococcus*) (SEQ ID NO: 18), *E. coli* (SEQ ID NO: 23), Sal (a *Salmonella*) (SEQ ID NO: 114).

On page 7, delete the tenth full paragraph, and replace this paragraph with the following:

Figure 19A-G presents an alignment of nucleotides of three β -glucuronidases; Staph (*Staphylococcus*) (SEQ ID NO: 115), E. coli (SEQ ID NO: 116), Sal (*Salmonella*) (SEQ ID NO: 113).

On page 8, delete the first full paragraph, and replace this paragraph with the following:

As used herein, a "secreted form of a microbial β -glucuronidase" refers to a microbial β -glucuronidase that is capable of being localized to an extracellular environment of a cell, including extracellular fluids, periplasm, or is membrane bound on the external face of a cell but is not an integral membrane protein. Some of the protein may be found intracellularly. The amino acid and nucleotide sequences of exemplary secreted β -glucuronidases are presented in Figures 1 and 16 and SEQ ID Nos.: 1, [2,] and 113. Secreted microbial GUS also encompasses variants of β -glucuronidase. A variant may be a portion of the secreted β -glucuronidase and/or have amino acid substitutions, insertions, and deletions, either found naturally as a polymorphic allele or constructed. A variant may also be a fusion of all or part of GUS with another protein.

On page 12, delete the third full paragraph, and replace this paragraph with the following:

A DNA sequence of the GUS gene contained in the insert of pRAJa17.1 is presented in Figure 1 and as SEQ ID No: 1. A schematic of the insert is presented in Figure 2. The β -glucuronidase gene contained in the insert is identified by similarity of the predicted amino acid sequence of an open reading frame to the *E. coli* and human β -glucuronidase amino acid sequences (Figure 5A). Overall, *Staphylococcus* β -

-glucuronidase has approximately 47-49% amino acid identity to *E. coli* GUS and to human GUS. An open reading frame of *Staphylococcus* GUS is 1854 bases, which would result in a protein that is 618 amino acids in length. The first methionine codon, however, is unlikely to encode the initiator methionine. Rather the second methionine codon is most likely the initiator methionine. Such a translated product is 602 amino acids long and is the sequence presented in Figures 3A-B and 4A-I. The assignment of the initiator methionine is based upon a consensus Shine-Dalgarno sequence found upstream of the second Met, but not the first Met, and alignment of the *Staphylococcus*, human, and *E. coli* GUS amino acid sequences. Furthermore, as shown herein, *Staphylococcus* GUS gene lacking sequence encoding the 16 amino acids is expressed in *E. coli* transfectants. In addition, the 16 amino acids (Met-Leu-Ile-Ile-Thr-Cys-Asn-His-Leu-His-Leu-Lys-Arg-Ser-Ala-Ile) SEQ ID No. 29 are not a canonical signal peptide sequence.

On page 13, delete the third full paragraph, and replace this paragraph with the following:

The DNA sequences of GUS genes from *Staphylococcus homini*, *Staphylococcus warneri*, *Thermotoga maritima* (TIGR *Thermotoga* database), *Enterobacter*, *Salmonella*, and *Pseudomonas* are presented in Figures 4A-J and SEQ ID Nos. 7-14. Predicted amino acid sequences are shown in Figures 3A-B and SEQ ID Nos. 2-6. The amino acid sequences are shown in alignment in Figures 5A-C. The signature peptide sequences for glycosyl hydrolases (Henrissat, *Biochem Soc Trans* 26:153, 1998; Henrissat B *et al.*, *FEBS Lett* 27:425, 1998) are located from amino acids 333 to 358 and from amino acids 406 to 420 (*Staphylococcus* numbering in Figures 3A and 5B). The catalytic nucleophile is Glu 344 (*Staphylococcus* numbering) (Wong *et al.*, *J. Biol Chem.* 18: 34057, 1998). Within these two signature regions, 17/26 and 8/15 residues are identical across the six presented sequences. At the non-identical positions, most of the sequences share an identical residue. Thus, the sequences are highly conserved in these regions (identity between *Staphylococcus* and each other GUS gene ranges from 65% to 100% in signature 1 and from 73% to 100% in signature 2) (see Figure 5B). In

contrast, between *Staphylococcus* and β -galactosidase, another glycosyl hydrolase that has signature sequences, identity is 46% in signature 1 and 73% in signature 2.

On page 18, delete the second full paragraph, and replace this paragraph with the following:

In other embodiments, variants may exhibit glucuronide binding activity without enzymatic activity or be directed to other cellular compartments, such as membrane or cytoplasm. Membrane-spanning amino acid sequences are generally hydrophobic and many examples of such sequences are well-known. These sequences may be spliced onto microbial secreted GUS by a variety of methods including conventional recombinant DNA techniques. Similarly, sequences that direct proteins to cytoplasm (*e.g.*, Lys-Asp-Glu-Leu (SEQ ID NO: 30)) may be added to the reference GUS, typically by recombinant DNA techniques.

On page 18, delete the third full paragraph, and replace this paragraph with the following:

In other embodiments, a fusion protein comprising GUS may be constructed from the nucleic acid molecule encoding microbial and another nucleic acid molecule. As will be appreciated, the fusion partner gene may contribute, within certain embodiments, a coding region. In preferred embodiments, microbial GUS is fused to avidin, streptavidin or an antibody. Thus, it may be desirable to use only the catalytic site of GUS (*e.g.*, amino acids 415-508 reference to *Staphylococcus* sequence). The choice of the fusion partner depends in part upon the desired application. The fusion partner may be used to alter specificity of GUS, provide a reporter function, provide a tag sequence for identification or purification protocols, and the like. The reporter or tag can be any protein that allows convenient and sensitive measurement or facilitates isolation of the gene product and does not interfere with the function of GUS. For example, green fluorescent protein and β -galactosidase are readily available as DNA sequences. A peptide tag is a short sequence, usually derived from a native protein, which is

recognized by an antibody or other molecule. Peptide tags include FLAG®, Glu-Glu tag (Chiron Corp., Emeryville, CA), KT3 tag (Chiron Corp.), T7 gene 10 tag (Invitrogen, La Jolla, CA), T7 major capsid protein tag (Novagen, Madison, WI), His₆ (SEQ ID NO: 117) (hexa-His), and HSV tag (Novagen). Besides tags, other types of proteins or peptides, such as glutathione-S-transferase may be used.

On page 19, delete the first full paragraph, and replace this paragraph with the following:

In other aspects of the present invention, isolated microbial glucuronidase proteins are provided. In one embodiment, GUS protein is expressed as a hexa-His (SEQ ID NO: 117) fusion protein and isolated by metal-containing chromatography, such as nickel-coupled beads. Briefly, a sequence encoding His₆ (SEQ ID NO: 117) is linked to a DNA sequence encoding a GUS. Although the His₆ (SEQ ID NO: 117) sequence can be positioned anywhere in the molecule, preferably it is linked at the 3' end immediately preceding the termination codon. The His-GUS fusion may be constructed by any of a variety of methods. A convenient method is amplification of the GUS gene using a downstream primer that contains the codons for His₆ (SEQ ID NO: 117).

On page 42, delete the second full paragraph, and replace this paragraph with the following:

The presence of GUS genes is established by amplification using degenerate oligonucleotides derived from a conserved region of the GUS gene. A pair of oligonucleotides is designed using an alignment of *E. coli* gusA and human GUS sequences. The primer T3-GUS-2F covers *E. coli* GUS amino acids 163-168 (DFFNYA) (SEQ ID NO: 31), while T7-GUS-5B covers the complementary sequence to amino acids 549-553 (WNFAD) (SEQ ID NO: 32). The full length of *E. coli* GUS is 603 amino acids. As shown in Table 1, amplification is not always successful, likely due to mismatching of the primers with template. Thus, a negative amplification does not necessarily signify that the microorganism lacks a GUS gene.

On page 49 delete the last paragraph under the heading "Example 6, Construction of a Codon Optimized Secreted β -Glucuronidase," and replace this paragraph with the following:

The *Staphylococcus* GUS gene is codon-optimized for expression in *E. coli* and in rice. Codon frequencies for each codon are determined by back translation using ecohigh codons for highly expressed genes of enteric bacteria. These ecohigh codon usages are available from GCG. The most frequently used codon for each amino acid is then chosen for synthesis. In addition, the polyadenylation signal, AATAAA, splice consensus sequences, ATTTA AGGT, and restriction sites that are found in polylinkers are eliminated. Other changes may be made to reduce potential secondary structure. To facilitate cloning in various vectors, four different 5' ends are synthesized: the first, called A0 (GT CGA CCC ATG GTA GAT CTG ACT AGT CTG TAC CCG) (SEQ ID NO: 51) uses a sequence comprising an *Nco* I (underlined), *Bgl* II (double underlined), and *Spe* I (italicized) sites. The Leu (CTG) codon is at amino acid 2 in Figures 3A-B. The second variant, called A1 (GTC GAC AGG AGT GCT ATC ATG CTG TAC CCG) (SEQ ID NO: 52), adds the native Shine/Dalgarno sequence 5' of the initiator Met (ATG) codon; the third, called AII, (GTC GAC AGG AGT GCT ACC ATG GTG TAC CCG) (SEQ ID NO: 53) adds a modified Shine/Dalgarno sequence 5' of the initiator Met codon such that a *Nco* I site is added; the fourth one, called AIII (GTC GAC AGG AGT GCT ACC ATG GTA GAT CTG TAC CCG) (SEQ ID NO: 54) adds a modified Shine/Dalgarno sequence 5' of the Leu (CTG) codon (residue 2) and *Nco* I and *Bgl* II sites.. All of these new 5' sequences contain a *Sa*I site at the extreme 5' end to facilitate construction and cloning. In certain embodiments, to facilitate protein purification, a sequence comprising a *Nhe* I, *Pml* I, and *Bst*E II sites (underlined) and encoding hexa-His amino acids joined at the 3' (COOH-terminus) of the gene.

gctagccatcaccatcaccatcacgtgtgaattggtgaccg (SEQ ID NO: 55)

SerSerHisHisHisHisHisHisVal * (SEQ ID NO: 56)

On page 50 Table 3, insert numbers in column four as follows:

Table 3

| Oligonucleotide | Size | Sequence | SEQ ID NO |
|--------------------------------|------|--|-----------|
| gusA ^{Stp} A-1-80T | 80 | TCGACCCATGGTAGATCTGACTAGTCTGTAC CCGATCAACACCGAGACCCGTGGCGTCTTC GACCTCAATGGCGTCTGGA | <u>57</u> |
| gusA ^{Stp} A-121-200B | 80 | GGATTTCTTGGTCACGCCAATGTCATTGTA ACTGCTTGGGACGGCCATACTAATAGTGTC GGTCAGCTTGCTTTCGTAC | <u>58</u> |
| gusA ^{Stp} A-161-240T | 80 | CCAAGCAGTTACAATGACATTGGCGTGACC AAGGAAATCCGCAACCATATCGGATATGTC TGGTACGAACGTGAGTTCAC | <u>59</u> |
| gusA ^{Stp} A-201-280B | 80 | GCGGAGCACGATACGCTGATCCTTCAGATA GGCCGGCACCCTGAACTCACGTTTCGTACCA GACATATCCGATATGGTTGC | <u>60</u> |
| gusA ^{Stp} A-241-320T | 80 | GGTGCCGGCCTATCTGAAGGATCAGCGTAT CGTGCTCCGCTTCGGCTCTGCAACTCACAA AGCAATTGTCTATGTCAATG | <u>61</u> |
| gusA ^{Stp} A-281-360B | 80 | AATGGCAGGAATCCGCCCTTGTGCTCCACG ACCAGCTCACCATTGACATAGACAATTGCT TTGTGAGTTGCAGAGCCGAA | <u>62</u> |
| gusA ^{Stp} A-321-400T | 80 | GTGAGCTGGTCGTGGAGCACAAGGGCGGAT TCCTGCCATTTCGAAGCGGAAATCAACAAC CGCTGCGTGATGGCATGAAT | <u>63</u> |
| gusA ^{Stp} A-361-460B | 100 | GTACAGCCCCACCGGTAGGGTGCTATCGTC GAGGATGTTGTCCACGGCGACGGTGACGCG ATTCATGCCATCACGCAGCGAGTTGTTGATT TCCGCTTCG | <u>64</u> |
| gusA ^{Stp} A-401-456T | 56 | CGCGTCACCGTCGCCGTGGACAACATCCTC GACGATAGCACCCCTACCGGTGGGGCT | <u>65</u> |
| gusA ^{Stp} A-41-120B | 80 | CACTTCTCTTCCAGTCCTTTCCCGTAGTCCA GCTTGAAGTTCCAGACGCCATTGAGGTCGA AGACGCCACGGGTCTCGGT | <u>66</u> |
| gusA ^{Stp} A-6-40B | 35 | TTGATCGGGTACAGACTAGTCAGATCTACC ATGGG | <u>67</u> |
| gusA ^{Stp} A-81-160T | 80 | ACTTCAAGCTGGACTACGGGAAAGGACTGG AAGAGAAGTGGTACGAAAGCAAGCTGACC GACACTATTAGTATGGCCGTC | <u>68</u> |
| gusA ^{Stp} B-1-80T | 80 | GTACAGCGAGCGCCACGAAGAGGGCCTCG GAAAAGTCATTTCGTAACAAGCCGAACCTCG ACTTCTTCAACTATGCAGGCC | <u>69</u> |
| gusA ^{Stp} B-121-200B | 80 | CTTTGCCTTGAAAGTCCACCGTATAGGTCAC AGTCCCGGTTGGGCCATTGAAGTCGGTCAC AACCGAGATGTCCTCGACG | <u>70</u> |
| gusA ^{Stp} B-161-240T | 80 | ACCGGGACTGTGACCTATACGGTGGACTTT CAAGGCAAAGCCGAGACCGTGAAAGTGTC GGTCGTGGATGAGGAAGGCAA | <u>71</u> |

| Oligonucleotide | Size | Sequence | SEQ ID NO |
|--------------------------------|------|---|-----------|
| gusA ^{Stp} B-201-280B | 80 | CTCCACGTTACCGCTCAGGCCCTCGGTGCTT GCGACCACTTTGCCTTCCTCATCCACGACCG ACACTTTCACGGTCTCGG | <u>72</u> |
| gusA ^{Stp} B-241-320T | 80 | AGTGGTCGCAAGCACCGAGGGCCTGAGCGG TAACGTGGAGATTCCGAATGTCATCCTCTG GGAACCACTGAACACGTATC | <u>73</u> |
| gusA ^{Stp} B-281-360B | 80 | GTCAGTCCGTCGTTACACAGTTCCACTTTGA TCTGGTAGAGATACGTGTTCAGTGGTTCCCA GAGGATGACATTCGGAAT | <u>74</u> |
| gusA ^{Stp} B-321-400T | 80 | TCTACCAGATCAAAGTGGAAGTGGTGAACG ACGGACTGACCATCGATGTCTATGAAGAGC CGTTCGGCGTGCGGACCGTG | <u>75</u> |
| gusA ^{Stp} B-361-440B | 80 | ACGGTTTGTGTTGATGAGGAAGTGGCGTC GTTGACTTCCACGGTCCGCACGCCGAACGG CTCTTCATAGACATCGATG | <u>76</u> |
| gusA ^{Stp} B-401-480T | 80 | GAAGTCAACGACGGCAAGTTCCTCATCAAC AACAAACCGTTCTACTTCAAGGGCTTTGGC AAACATGAGGACACTCCTAT | <u>77</u> |
| gusA ^{Stp} B-41-120B | 80 | TACGTAAACGGGGTTCGTGTAGATTTTCACC GGACGGTGCAGGCCTGCATAGTTGAAGAAG TCGAAGTTCGGCTTGTTACG | <u>78</u> |
| gusA ^{Stp} B-441-520B | 80 | ATCCATCACATTGCTCGCTTCGTTAAAGCCA CGGCCGTTGATAGGAGTGTCTCATGTTTGC CAAAGCCCTTGAAGTAGA | <u>79</u> |
| gusA ^{Stp} B-481-555T | 75 | CAACGGCCGTGGCTTTAACGAAGCGAGCAA TGTGATGGATTTCAATATCCTCAAATGGATC GGCGCCAACAGCTT | <u>80</u> |
| gusA ^{Stp} B-5-40B | 36 | AATGACTTTTCCGAGGCCCTCTTCGTGGCGC TCGCT | <u>81</u> |
| gusA ^{Stp} B-521-559B | 39 | CCGGAAGCTGTTGGCGCCGATCCATTTGAG GATATTGAA | <u>82</u> |
| gusA ^{Stp} B-81-160T | 80 | TGCACCGTCCGGTGAAAATCTACACGACCC CGTTTACGTACGTCGAGGACATCTCGGTTGT GACCGACTTCAATGGCCCA | <u>83</u> |
| gusA ^{Stp} C-1-80T | 80 | CCGGACCGCACACTATCCGTAATCTGAAGA GTTGATGCGTCTTGGCGATCGCGAGGGTCT GGTCGTGATCGACGAGACTC | <u>84</u> |
| gusA ^{Stp} C-121-200B | 80 | GTTACGGAGAACGTCTTGATGGTGCTCAA ACGTCCGAATCTTCTCCAGGTAATGACGC GCTCGCTGCCTTCGCCGAGT | <u>85</u> |
| gusA ^{Stp} C-161-240T | 80 | ATTCGGACGTTTGAGCACCATCAAGACGTT CTCCGTGAAGTGGTGTCTCGTGACAAGAAC CATCCAAGCGTCGTGATGTG | <u>86</u> |
| gusA ^{Stp} C-201-280B | 80 | CGCGCCCTCTTCTCAGTCGCCGCCTCGTTG GCGATGCTCCACATCACGACGCTTGGATGG TTCTTGTCACGAGACACCA | <u>87</u> |

| Oligonucleotide | Size | Sequence | SEQ ID NO |
|--------------------------------|------|---|------------|
| gusA ^{Stp} C-241-320T | 80 | GAGCATCGCCAACGAGGCGGCGACTGAGG AAGAGGGCGCGTACGAGTACTTCAAGCCGT TGGTGGAGCTGACCAAGGAAC | <u>88</u> |
| gusA ^{Stp} C-281-360B | 80 | ACAAACAGCACGATCGTGACCGGACGCTTC TGTGGGTCGAGTTCTTGGTCAGCTCCACCA ACGGCTTGAAGTACTCGTA | <u>89</u> |
| gusA ^{Stp} C-321-400T | 80 | TCGACCCACAGAAGCGTCCGGTCACGATCG TGCTGTTTGTGATGGCTACCCCGGAGACGG ACAAAGTCGCCGAAGTATT | <u>90</u> |
| gusA ^{Stp} C-361-440B | 80 | CGAAGTACCATCCGTTATAGCGATTGAGCG CGATGACGTCAATCAGTTCGGCGACTTTGTC CGTCTCCGGGGTAGCCATC | <u>91</u> |
| gusA ^{Stp} C-401-489T | 89 | GACGTCATCGCGCTCAATCGCTATAACGGA TGGTACTTCGATGGCGGTGATCTCGAAGCG GCCAAAGTCCATCTCCGCCAGGAATTTCA | <u>92</u> |
| gusA ^{Stp} C-41-120B | 80 | CCCGTGGTGGCCATGAAGTTGAGGTGCACG CCAAGTCCCGAGTCTCGTCGATCACGACC AGACCCTCGCGATCCGCAAG | <u>93</u> |
| gusA ^{Stp} C-441-493B | 53 | CGCGTGAAATTCCTGGCGGAGATGGACTTT GGCCGCTTCGAGATCACCGCCAT | <u>94</u> |
| gusA ^{Stp} C-5-40B | 36 | ACGCATCAACTCTTCAGAGTACGGATAGTG TGCGGT | <u>95</u> |
| gusA ^{Stp} C-81-160T | 80 | CGGCAGTTGGCGTGACCTCAACTTCATGG CCACCACGGGACTCGGCGAAGGCAGCGAG CGCGTCAGTACCTGGGAGAAG | <u>96</u> |
| gusA ^{Stp} D-1-80T | 80 | CGCGTGGAACAAGCGTTGCCAGGAAAGCC GATCATGATCACTGAGTACGGCGCAGACAC CGTTGCGGGCTTTACGACA | <u>97</u> |
| gusA ^{Stp} D-121-200B | 80 | TCGCGAAGTCCGCGAAGTTCCACGCTTGCT CACCCACGAAGTTCTCAAACCTCATCGAACA CGACGTGGTTCGCCTGGTAG | <u>98</u> |
| gusA ^{Stp} D-161-240T | 80 | TTCGTGGGTGAGCAAGCGTGGAAGTTTCGCG GACTTCGCGACCTCTCAGGGCGTGATGCGC GTCCAAGGAAACAAGAAGGG | <u>99</u> |
| gusA ^{Stp} D-201-280B | 80 | GTGCGCGGCGAGCTTCGGCTTGCGGTCACG AGTGAACACGCCCTTCTTGTTTCTTGACG CGCATCACGCCCTGAGAGG | <u>100</u> |
| gusA ^{Stp} D-241-320T | 80 | CGTGTTCACTCGTGACCGCAAGCCGAAGCT CGCCGCGCACGTCTTTCGCGAGCGCTGGAC CAACATTCCAGATTTTCGGCT | <u>101</u> |
| gusA ^{Stp} D-281-369B | 89 | CGGTCACCAATTCACACGTGATGGTGATGG TGATGGCTAGCGTTCTTGAGCCGAAATCTG GAATGTTGGTCCAGCGCTCGCGAAAGAC | <u>102</u> |
| gusA ^{Stp} D-321-373T | 53 | ACAAGAACGCTAGCCATCACCATCACCATC ACGTGTGAATTGGTGACCGGGCC | <u>103</u> |

| Oligonucleotide | Size | Sequence | SEQ ID NO |
|-------------------------------|------|--|------------|
| gusA ^{Stp} D-41-120B | 80 | TACTCGACTTGATATTCCTCGGTGAACATCA CTGGATCAATGTCGTGAAAGCCCGCAACGG TGTCTGCGCCGTACTCAGT | <u>104</u> |
| gusA ^{Stp} D-5-40B | 36 | GATCATGATCGGCTTTCCTGGGCAACGCTTG TTCCA | <u>105</u> |
| gusA ^{Stp} D-81-160T | 80 | TTGATCCAGTGATGTTACCGAGGAATATC AAGTCGAGTACTACCAGGCGAACCACGTCG TGTTTCGATGAGTTTGAGAAC | <u>106</u> |

On page 55 delete the paragraph stating with "Invertase signal sequence:" and replace this paragraph with the following:

Invertase signal sequence:

ATGCTTTTGC AAGCCTTCCT TTTCCTTTTG GCTGGTTTTG CAGCCAAAAT
ATCTGCAATG (SEQ ID NO: 107)

Mat alpha signal sequence:

ATGAGATTTTC CTTCAATTTT TACTGCAGTT TTATTCGCAG CATCCTCCGC
ATTAGCTGCT CCAGTCAACA CTACAACAGA AGATGAAACG GCACAAATTC
CGGCTGAAGC TGTCATCGGT TACTTAGATT TAGAAGGGGA TTTCGATGTT
GCTGTTTTGC CATTTTCCAA CAGCACAAAT AACGGGTTAT TGTTTATAAA
TACTACTATT GCCAGCATTG CTGCTAAAGA AGAAGGGGTA TCTTTGGATA
AAAGAGAG (SEQ ID NO: 108)

Extensin signal sequence

CATGGGAAAA ATGGCTTCTC TATTTGCCAC ATTTTATAGTG GTTTTAGTGT
CACTTAGCTT AGCTTCTGAA AGCTCAGCAA ATTATCAA (SEQ ID NO. 109)

GRP signal sequence

CATGGCTACT ACTAAGCATT TGGCTCTTGC CATCCTTGTC CTCCTTAGCA
TTGGTATGAC CACCAGTGCA AGAACCCTCC TA (SEQ ID NO. 110)

On page 56, delete the fourth full paragraph, and replace this paragraph with the following:

The oligonucleotides Asn-T, 5'-A TTC CTG CCA TTC GAG GCG GAA ATC NNG
AAC TCG CTG CGT GAT-3' (SEQ ID No. 111) and Asn-B, 5'-ATC ACG CAG CGA GTT

CNN GAT TTC CGC CTC GAA TGG CAG GAA T-3' (SEQ ID No. 112), are used in the "quikchange" mutagenesis method by Stratagene (La Jolla, CA) to randomize the first two nucleotides of the Asn 118 codon, AAC. The third base is changed to a G nucleotide, so that reversion to Asn is not possible. In theory a total of 13 different amino acids are created at position 118.